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# Candidate gene associations reveal sex-specific Graves' disease risk alleles among Chinese Han populations

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## Abstract

**Background:** With several susceptibility single nucleotide polymorphisms identified by case–control association studies, Graves' disease is one of the most common forms of autoimmune thyroid disease. In this study, we aimed to determine whether any observed differences in genetic associations are influenced by sex in Chinese Han populations.

**Methods:** A total of 8,835 patients with Graves' disease and 9,936 sex-matched healthy controls were enrolled in the study. Confirmed by a two-staged association analysis, sex-specific analyses among 20 Graves' disease susceptibility loci were conducted.

**Results:** A significant sex-gene interaction was detected primarily at rs5912838 on Xq21.1 between the *GPR174* and *ITM2A* genes, whereby male Graves' disease patients possessed a significantly higher frequency of risk alleles than their female counterparts. Interestingly, compared to women, male patients with Graves' disease had a higher cumulative genetic risk and higher persistent thyroid stimulating hormone receptor antibody-positive rate after receiving antithyroid drug therapy for at least 1 year.

**Conclusion:** The findings of this study suggest the existence of one potential sex-specific Graves' disease variant on Xq21.1. This could increase our understanding of the pivotal mechanism behind Graves' disease and ultimately aid in identifying possible therapeutic targets.

**KEYWORDS**
association analysis, Graves' disease, sex-specific, single nucleotide polymorphism

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## 1 | INTRODUCTION

Graves' disease (GD) is one of the most common forms of autoimmune thyroid disease with an annual incidence of 20–50 cases per 100,000 persons, is characterized by hyperthyroidism and thyroid-stimulating hormone receptor autoantibodies (TRAb) in the serum (Zimmermann & Boelaert, 2015). Currently understood to be multifactorial, the etiology of GD is due to a complex interplay between specific susceptibility genes and environmental exposure, with family history and twin studies having identified the pivotal role that genetic factors play in GD (Brix, Christensen, Holm, Harvald, & Hegedüs, 1998; Brix & Hegedüs, 2012). Previous studies have identified a series of GD susceptibility genes in different ethnic groups. These susceptibility genes can be categorized as either thyroid specific (thyroglobulin, *TG*; thyroid stimulating hormone receptor, *TSHR*) or immune-modulating (forkhead box P3, *FOXP3*; interleukin 2 receptor subunit alpha, *CD25*; CD40 molecule, *CD40*; cytotoxic T-lymphocyte associated protein 4, *CTLA-4*; major histocompatibility complex, class II, DR beta 3, *HLA-DR3*; Protein Tyrosine Phosphatase-22, *PTPN22*; secretoglobin family 3A member 2, *SCGB3A2*; ribonuclease T2, *RNASET2*). To date, 20 single nucleotide polymorphisms (SNPs) located in 15 regions have been validated in our previous study in the Chinese Han population (Chu et al., 2011; Liu et al., 2018; Zhao, Xue, et al., 2013). For instance, in our first genome-wide association study (GWAS), two new susceptibility loci for GD (rs9355610 in *RNASET2-FGFR1OP* region at 6q27 and rs6832151 in intergenic region at 4p14) were identified, and four previously reported loci (in *HLA*, *TSHR*, *CTLA4,* and *FCRL3*) were confirmed (Chu et al., 2011). In recent study from our group, we identified five previously undetected GD risk loci (rs5912838 between *GPR174* and *ITM2A* at Xq21.1, rs1456988 at 14q32.2, rs229527 and rs2284038 at 22q12.3–13.1 and the rs1265883 in *SLAMF6* region at 1q23.2) and confirmed controversial loci ( in *TG*, *ABO*, and *BACH2*) in the Chinese Han population (Zhao, Xue, et al., 2013). Yet, sex differences have not been integrated into these studies.

Sex differences exist in various common diseases in humans, such as asthma (Postma, 2007), cardiovascular (Choi & McLaughlin, 2007), and autoimmune diseases (Rubtsova, Marrack, & Rubtsov, 2015). These differences between males and females are known to affect the incidence and progression of these diseases. The prevalence of GD shows an intriguing sex-specific architecture in which females are 5–10 times more likely to develop the disease than men (Brent, 2008). In a retrospective study in the UK, it was suggested that males suffered biochemically worse hyperthyroidism with less severe symptoms, responded poorly to a single dose of radioiodine and had a lower remission rate than females after a course of antithyroid medication (Allahabadia et al., 2000). It has become clear that it is critical to understand sex differences in GD to optimize prevention, diagnosis, and therapeutic intervention for both sexes. Typically, sex differences have been attributed not only to the variation in the sex-related hormone levels between males and females, but also to the genetic contribution of the sex chromosomes (Amur, Parekh, & Mummaneni, 2012). Broadly speaking, X-linked dosage differences potentially explain the sex-specific genetic architecture and phenotypes of some common diseases. Supplementing this statement is the increasing evidence suggesting that variation in autosomal genes can have sex-specific phenotypic effects in humans (Gautam, Afanador, Abebe, López, & Mersha, 2019; Hughes et al., 2012; Ng et al., 2016). Not including the influence of the sex chromosomes, the autosomal genome generates phenotypic sexual dimorphism through sex-specific differences in gene regulation and gene expression rather than gene content (Ellegren & Parsch, 2007). Moreover, with the advent of GWASs, SNPs can be systematically analyzed in depth to detect sex differences in disease association and effect (Liu, Schaub, Sirota, & Butte, 2012). Indeed, identifying sex-specific variants in large GWASs provides an increased understanding of the pathogenesis and genetics of diseases and broadens the repertoire of potential drug targets.

Through the sharing of susceptibility loci and the direction of gene effect (whether the associated variant is predisposing or protective), the genetic architecture of GD is similar to other autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, ankylosing spondylitis, type 1 diabetes, and Crohn's disease (Sirota, Schaub, Batzoglou, Robinson, & Butte, 2009). For example, genetic polymorphisms in the *Protein Tyrosine Phosphatase-22* (*PTPN22*) have reproducibly shown associations with systemic lupus erythematosus (SLE), GD, and rheumatoid arthritis (RA) (Shoenfeld, Tincani, & Gershwin, 2012). Notably, while RA and GD share susceptibility loci, the direction of effect differs (Boelaert et al., 2010). A recent analysis of autosomal genes revealed gene-sex interactions and higher total genetic risk in men with SLE (Hughes et al., 2012), which aroused our interest in the study of the gene-sex interactions of GD.

Collectively, these data suggest that genetic architecture may contribute to sexual dimorphism in autoimmune diseases. However, few studies have been conducted with larger sample sizes to verify sex-specific genetic effects in GD. Consequently, the objective of our study was to characterize the sex-specific genetic architecture of GD in the Chinese Han population.

## 2 | MATERIALS AND METHODS

### 2.1 | Study subjects and ethical compliance

Subjects were selected from the previously used database for inherited thyroid diseases (Zhao, Xue, et al., 2013). In the discovery and replication stages, 1,442 GD patients and 1,468

sex-matched controls, and 7,993 GD patients and 8,468 sex-matched controls, were recruited, respectively. Sample sets obtained for the current study were described in Table 1. A diagnosis of GD was based on documented clinical and biochemical evidence of GD, a diffuse goiter, and the presence of at least one of the following: a positive TRAb test, diffusely increased ^{131}I uptake in the thyroid gland, or exophthalmos (Chu et al., 2011; Zhao, Xue, et al., 2013). Control subjects had no GD and no family history of GD or any other autoimmune disorders. The control group consisted of sex-matched individuals older than 35 years. This age was chosen to reduce the possibility that the individuals would develop GD at a later stage (Chu et al., 2011). The definition of "TRAb positive-persisting" (pTRAb[+]), and "TRAb negative-conversing" (pTRAb[−]) were according to a previous description (Ma et al., 2019). Written informed consent was obtained from each participant enrolled in the study. The study protocol conformed to ethical guidelines and was approved by the local ethics committee. The population structure of the case–control samples was evaluated via principal component and multidimensional scaling analyses, and showed no evidence of population stratification in the study cohort (Chu et al., 2011). In present study, to evaluate the population structure in samples, principal component analysis (PCA, Figure S1) was performed by SmartPCA16 (Price et al., 2006). No significant population structures differences were observed in our current cohorts, and males and females were labeled with different color.

## 2.2 | SNP selection

The SNPs were selected from results obtained from several previous genetic association studies in GD (Chu et al., 2011; Du et al., 2014; Liu, Wang, et al., 2014; Liu, Yang, et al., 2014; Liu et al., 2018; Ye et al., 2017; Zhao, Liu, et al., 2013; Zhao, Xue, et al., 2013). These case–control association studies identified new risk loci for GD and confirmed controversial loci in the Chinese Han population. Criteria for reaching genome-wide significance required a $p$-value of less than $5.0 \times 10^{-8}$ and independent

GD susceptibility variants in the replication and combined populations. Ultimately, 20 SNPs were subsequently selected from 15 genomic regions; their association with GD is reflected in Table S1.

## 2.3 | Statistical analysis

A $p$-value of less than 0.05 was considered to be significantly different in the discovery stage to avoid eliminating any possible SNP. All association analyses were run on PLINK (v1.07, URL: http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell et al., 2007) with each case–control group tested for Hardy–Weinberg equilibrium. Risk allele frequencies (RAF) of each SNP were compared between GD patients and controls using the Cochran–Armitage trend test. The odds ratio (OR) and 95% confidence intervals (CIs) were calculated using logistic regression. Sex-specific association analyses were performed by comparing allele frequencies between cases and controls from each sex separately, followed by case–case analysis. Following stratified analysis, a standard case–control association test was performed using a Cochran–Mantel–Haenszel statistic that tests for SNP-disease association conditional on the clustering. The Breslow–Day test was utilized to examine heterogeneity among subgroups. The priori criterion for correction following multiple testing was set at $p < .0025$ (a Bonferroni correction for the number of SNPs examined; 0.05/20). For variation in autosomal genes, a sex × SNP interaction term was assessed using a logistic regression approach as implemented in PLINK. Differences between males and females were compared using an independent sample $t$-test or chi-squared test.

## 2.4 | Weighted genetic risk scores

The aggregate genetic risk was examined in a sex-specific manner by calculating weighted genetic risk scores (wGRSs) for individuals with 100% genotype success rate. The 20 confirmed GD susceptibility SNPs in a Chinese Han population were all included when calculating the wGRSs. ORs used to calculate the risk score in each sex were those obtained in the sex-specific association analyses in male and female populations, respectively. In this study, the wGRS for each individual was calculated by multiplying the number of risk alleles at each locus (0, 1, and 2) by the weight for that SNP (the natural log of the OR for each sex-specific association), and then summing the products. The formula of final wGRS was previously described (Ma et al., 2019), in short, dividing all values by the sum of the weight and then multiplying by the total number of SNPs. The power of the wGRS predictors was evaluated by receiver operating characteristic (ROC) curves. Then calculating the area under the curve (AUC) and 95% CI of AUCs in each data set. Differences in the predictive performance of different

**TABLE 1** Description of the sample sets in the current study

| Genotyping stage | Disease status | No. | Sex ratio (M/F) |
|---|---|---|---|
| Discovery | GD | 1,442 | 335/1107 |
| | Control | 1,468 | 359/1109 |
| Replication | GD | 7,993 | 1887/6104 |
| | Control | 8,468 | 1967/6501 |
| Combined | GD | 8,458 | 2001/6350 |
| | Control | 9,158 | 2520/6613 |

Abbreviations: F, female; GD, Graves' disease; M, male; No., number.

genetic models were tested using pROC package in R (version 3.4.3). Finally, risk scores were calculated for a total of 1,290 men and 3,607 women with GD.

# 3 | RESULTS

## 3.1 | Sex-specific genetic association analyses

First, GWAS data from previous studies were reevaluated. Twenty SNPs located in 15 chromosomal regions were found to show unequivocal evidence of association with GD when using a genome-wide significance threshold of $p = 5 \times 10^{-8}$ (Table S1). The association was confirmed by a two-staged analysis. In the discovery stage, there were 1,442 GD cases and 1,468 sex-matched controls. Among the GD cases, 1,107 (76.8%) were female and 335 (23.2%) were male. There were 1,109 (75.5%) females to 359 (24.5%) males among the control participants (Table 1). In the replication stage, 7,993 GD cases and 8,468 sex-matched controls were included (Table 1).

Second, to investigate the sex bias in the association of the SNPs with GD, we performed separate case–control association tests in men and women. In the discovery stage (Table S2), all 20 SNPs were significantly associated with GD in females ($P_{\text{discovery}} < 0.05$). In contrast to this, only 12 SNPs were significantly associated with GD in males ($P_{\text{discovery}} < 0.05$). The RAF between men and women with GD (Table S2) were subsequently compared. Four SNPs (rs1521, rs6457617, rs505922, and rs5912838) showed a significant difference between female and male GD patients ($P_{\text{sex}} < 0.05$, Table S2).

In order to include the possible sex-specific loci, all SNPs were replicated in a cohort of 7,993 GD cases and 8,468 sex-matched controls using the same method (Table S3). Consequently, three of the four SNPs (rs1521, rs6457617, and rs5912838) still showed significant differences between male and female GD patients with a priori threshold of $P_{\text{sex}} < 0.0025$. No significant genetic heterogeneity in the two GD subgroups was found for rs505922 ($p = .8,377$, OR = 0.99, 95% CI = 0.93–1.06; Table S3). Given the small discovery stage sample size, rs4947296 was considered individually as a candidate marker for a sex-specific locus after the replication stage. This analysis showed significant allelic differences between men and women with GD ($p = .0023$, OR = 0.84, 95% CI = 0.75–0.94; Table S3). A combined analysis was consequently performed in 8,458 patients with GD and 9,158 controls. All four SNPs (rs4947296, rs1521, rs6457617, and rs5912838) showed significant differences (Table S4). Thus, subsequent research was concentrated on these four sex-specific candidate loci.

## 3.2 | Identification of sex-specific loci associated with GD

Among the four SNPs (rs4947296, rs1521, rs6457617, and rs5912838), sex differences were again observed when the analyses were conducted separately in males and females (Table 2). In the female subgroup, these SNPs were significantly associated with GD, yielding ORs of 1.66 for rs4947296, 1.74 for rs1521, 1.29 for rs6457617, and 1.26 for rs5912838 (Table 2). Comparatively, in males with GD, three SNPs carried strong ORs of 1.89 for rs1521, 1.49 for rs6457617, and 1.65 for rs5912838, respectively. The rs4947296 SNP carried a weaker OR of 1.53 in males than females (Table 2). Interestingly and as shown in Table S4, the frequencies of the risk alleles in the three loci of GD patients were significantly higher in men than in

**TABLE 2** Genetic associations in men and women with GD compared to healthy male and female controls of combined data

| SNP ID | Annotated genes | Risk Allele | Female | | | | Male | | | | |
| | | | RAF | | OR (95% CI) | $P$ | RAF | | OR (95% CI) | $P$ | $P_{BD}$ |
| | | | Case | Control | | | Case | Control | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| rs4947296 | C6orf15/ PBMUCL1 | C | 0.21 | 0.14 | 1.66 (1.53–1.80) | $2.18 \times 10^{-33}$ | 0.24 | 0.17 | 1.53 (1.33–1.75) | $1.83 \times 10^{-9}$ | .2976 |
| rs1521 | MICA/HLA-B | T | 0.87 | 0.80 | 1.74 (1.61–1.89) | $7.27 \times 10^{-39}$ | 0.90 | 0.83 | 1.89 (1.61–2.21) | $7.61 \times 10^{-15}$ | .3749 |
| rs6457617 | HLA-DQB1/ HLA-DQA2 | T | 0.52 | 0.46 | 1.29 (1.22–1.37) | $6.49 \times 10^{-17}$ | 0.59 | 0.49 | 1.49 (1.33–1.67) | $3.25 \times 10^{-13}$ | .02109 |
| rs5912838 | GPR174/ ITM2A | A | 0.64 | 0.58 | 1.26 (1.21–1.33) | $5.44 \times 10^{-22}$ | 0.69 | 0.58 | 1.65 (1.46–1.85) | $9.47 \times 10^{-17}$ | $2.67 \times 10^{-5}$ |

*Note:* All *p*-values were corrected using the Bonferroni method ($p = .0025$). $P_{BD}$: the Breslow–Day test was used to test for the heterogeneity for associations between men and women.

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio for the risk allele; RAF, risk allele frequency; SNP, single nucleotide polymorphism.

women (rs1521, $OR_{female-male} = 0.75$, 95% CI 0.65 to 0.86, $p = 4.17 \times 10^{-5}$; rs6457617, $OR_{female-male} = 0.78$, 95% CI 0.71 to 0.85, $p = 5.23 \times 10^{-9}$; and rs5912838, $OR_{female-male} = 0.77$, 95% CI 0.70 to 0.85, $p = 1.79 \times 10^{-7}$). This was also the case for rs4947296 ($OR_{female-male} = 0.85$, 95% CI 0.77 to 0.94, $p = .0017$), which is contrary to what would be expected. Thus, the difference of risk allele frequencies between male and female controls were investigated (Table 3). It was found that rs4947296 and rs1521, rather than rs6457617 and rs5912838, exhibited significant differences for the RAFs between the two sexes in the control groups ($p = .0002$ and $p = .0005$, respectively). Therefore, when excluding the effects of the control groups, it is believed that the significant sex-associated differences in patients with GD are linked to rs6457617 and rs5912838. This trend was further examined by performing the Breslow–Day test to assess homogeneity between male and female subgroups (Table 2). Significant allelic differences between men and women with GD were observed for both rs6457617 and rs5912838 ($P_{BD} = 0.02109$, and $P_{BD} = 2.67 \times 10^{-5}$, respectively; Table 2). However, the rs6457617 SNP did not indicate significance following Bonferroni correction. Sex × SNP interaction analysis supported the findings showing evidence for interactions between three autosomal susceptibility loci and sex in patients with GD ($p = .1505$ for rs4947296; $p = .8912$ for rs1521; and $p = .02367$ for rs6457617, respectively).

## 3.3 | Sex-specific differences in cumulative genetic risk and manifestations

Sex-specific differences in overall GD genetic risk between men and women were further investigated by calculating wGRS for GD in each individual. Scores were calculated based on the ORs obtained in the sex-specific case–control association analysis. The ability of the model to discriminate GD patients from the general population was assessed by ROC curves. As a result, the AUC achieved 0.69 (95% CI: 0.68–0.69), the sensitivity was 0.69 and specificity was 0.59 in females, while in males, the AUC was 0.70 (95% CI: 0.68–0.70), the sensitivity was 0.73 and specificity was 0.59 (Table 4). Using a Student's $t$ test it was observed that, on average, male patients have a significantly higher genetic risk than female patients (mean ± SD, 20.70 ± 3.42 for males, 20.06 ± 3.20 for females, $p = 4.67 \times 10^{-9}$; Table 4, Figure 1). While not unexpected, it was interesting to observe a contrary tendency upon removal of rs5912838 and its corresponding effect specific to men. Moreover, and as shown in Figure 2, male patients consistently had a higher pTRAb$^+$ rate than females ($p = .013$). These findings suggested that the male-specific locus may explain the characteristics of clinical manifestations in male patients with GD.

## 4 | DISCUSSION

Since the X chromosome was excluded in previous GWASs, the contribution of X-chromosome genes to sex differences in GD may have been underestimated. In this study, sex-specific genetic differences in GD have been reported by expanding a recent GWAS of GD to include the X chromosome. Interestingly, the most significant sex-gene interaction was observed at rs5912838 on Xq21.1, notwithstanding we could not completely exclude the possibility of the effect of population stratification between males and females. Importantly, it was demonstrated in patients with GD that men have a higher cumulative genetic risk than women. Notably, there was a higher pTRAb$^+$ rate in male patients with GD than females.

**TABLE 3** Sex–gene disparities between men and women with or without GD

| | | | GD | | | | Control | | | |
| | | | RAF | | | | RAF | | | |
| SNP ID | Annotated genes | Risk Allele | Female | Male | OR (95% CI) | P | Female | Male | OR (95% CI) | P |
|---|---|---|---|---|---|---|---|---|---|---|
| rs4947296 | C6orf15/ PBMUCL1 | C | 0.21 | 0.24 | 0.85 (0.77–0.94) | .0017 | 0.14 | 0.17 | 0.80 (0.71–0.90) | .000168 |
| rs1521 | MICA/HLA-B | T | 0.87 | 0.90 | 0.75 (0.65–0.86) | $4.17 \times 10^{-5}$ | 0.80 | 0.83 | 0.81 (0.73–0.91) | .000522 |
| rs6457617 | HLA-DQB1/ HLA-DQA2 | T | 0.52 | 0.59 | 0.78 (0.71–0.85) | $5.23 \times 10^{-9}$ | 0.46 | 0.49 | 0.90 (0.82–0.98) | .01551 |
| rs5912838 | GPR174/ ITM2A | A | 0.64 | 0.69 | 0.77 (0.70–0.85) | $1.79 \times 10^{-7}$ | 0.58 | 0.58 | 1 ($1.25 \times 10^{-26} - 7.99 \times 10^{25}$) | 1 |

*Note:* All p-values were corrected using the Bonferroni method ($p = .0025$).

Abbreviations: 95% CI, 95% confidence intervalGD: Graves' disease;OR, odds ratio for the risk allele; RAF, risk allele frequency; SNP, single nucleotide polymorphism.

**TABLE 4** The parameters of wGRS model predict GD in males and females

| Group | wGRS | AUC (95%CI) | OR (95%CI) | _P_ | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| Males | 20.70 ± 3.42 | 0.70 (0.68–0.70) | 1.24 (1.20–1.27) | $4.31 \times 10^{-57}$ | 0.73 | 0.59 |
| Females | 20.06 ± 3.20 | 0.69 (0.68–0.69) | 1.23 (1.21–1.25) | $3.14 \times 10^{-161}$ | 0.69 | 0.59 |

Abbreviations: 95% CI, 95% confidence interval; AUC, area under the receiver operator characteristic curve; OR, odds ratio for the risk allele; wGRS, weighted genetic risk score, presenting by the means and standard deviations (S.D.) of each data set.

Sexual disparities at the X chromosome locus appear to account for the largest proportion of genetic variation in overall risk between men and women with GD. One independent genetic susceptibility locus within the Xq21.1 region was tested and provided robust evidence for a sex-gene interaction in this locus. Unexpectedly, male patients have significantly higher risk allele frequencies than females in this susceptibility locus. Under normal conditions, a balanced gene expression dosage between males (XY) and females (XX) is achieved by X chromosome inactivation (XCI). However, a number of genes escape XCI in women. Differences in the identity and distribution of escaped genes suggest a role for these genes in the evolution of sex differences in specific phenotypes. The rs5912838 SNP is located between *GPR174* and *ITM2A*. Previous studies have revealed that *ITM2A*, instead of *GPR174*, was found to escape from XCI in peripheral blood mononuclear cells (PBMCs) (Zhao, Xue, et al., 2013). However, XCI alone cannot explain the observation that men were found to have a higher prevalence of the rs5912838 risk allele than women. Encoding a protein belonging to the G protein-coupled receptor superfamily, little is known about *GPR174*. A recent study found that environmental factors trigger the development of GD by interacting with genetic variants such as the susceptibility associated SNP rs3827440 in *GPR174* (Ye et al., 2017). Therefore, it is proposed that *GPR174* or additional, undetected sequence elements in this critical region may be contributing to male-specific GD susceptibility.

In calculating wGRS, the number of risk alleles inherited for each individual in a given population is counted. The outcomes thereof provide a quantitative measure of genetic risk (Chatterjee, Shi, & García-Closas, 2016). Indeed, developing sex-specific GRS will reduce misclassification and improve diagnostic precision. For example, female-specific GRS have been tested in relation to natural menopause (Pasquale et al., 2017), polycystic ovary syndrome (Lee, Oh, Sung, & Chung, 2016), multiple sclerosis (Xia et al., 2017), and breast cancer (Vachon et al., 2015). Similarly, male-specific GRS has been tested for prostate cancer (Helfand, Kearns, Conran, & Xu) and systemic lupus erythematosus (Hughes et al., 2012). To date, almost all GD studies have failed to examine sex-specific effects in GRS. As demonstrated by increased weighted genetic risk scores, in 20 previously established risk loci for GD, men who develop GD possess a higher aggregate genetic risk. The increased genetic risk among men may account for their reduced overall incidence of GD, while simultaneously reinforcing the molecular processes underlying the disease and its potential to have more severe disease manifestations. Indeed, this hypothesis was supported by comparing the pTRAb$^+$ rate between males and female patients. The positive ratio was found to be higher in men. Additionally, it has been reported that the recurrence risk was higher in TRAb-positive GD patients at the



**FIGURE 1** Distribution curves for weighted genetic risk scores for GD in men (blue) and women (red) showing a higher genetic risk in men than in women ($p = 4.67 \times 10^{-9}$). Sex-specific ORs (Table 2) were used to calculate the weighted genetic risk score (wGRS) in male and female patients
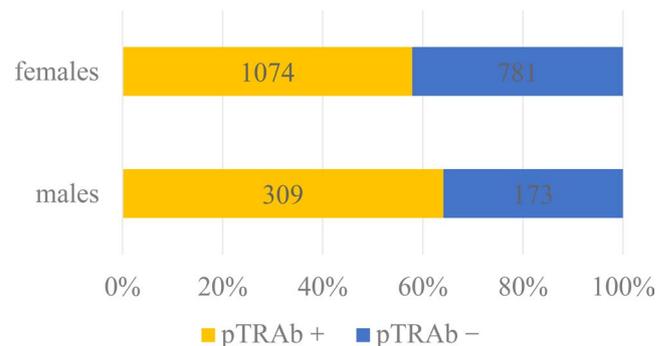


**FIGURE 2** A higher persistent TRAb-positive rate was found in male patients when compared to their female counterparts following antithyroid drug treatment for no less than 1-year ($p = .013$). The 100% stacked column chart was used to show pTRAb$^+$ (yellow) and pTRAb$^-$ (blue) ratio in males or females separately. The breadth of each legend represents the percentage value. The absolute count of people is shown in each stack. pTRAb$^+$, persistent thyroid stimulating hormone receptor antibody-positive; pTRAb$^-$, persistent thyroid stimulating hormone receptor antibody-negative.

time of drug withdrawal (Wang et al., 2013). As is the case for the acquired immune deficiency syndrome, a single nucleotide polymorphism located at Xq21.1 is associated with disease progression (Siddiqui et al., 2009). However, whether or not these male-specific loci are in association with difference in disease severity between men and women remain to be further tested.

A case–control study for Graves' disease in a Hong Kong Chinese population revealed that *HLA-646, DR9,* and *DQB1\*0,303* were associated only in males (Cavan et al., 1994). However, in this study, the rs6457617 SNP in the MHC region between *HLA-DQB1* and *HLA-DQA2* had a less significant *P*-value for a male-specific association with GD. Significance did not remain after adjustment for multiple testing using Bonferroni correction. Notably, our association analyses were performed separately for each sex. This reduced the sample size to conduct sex-specific analysis by approximately 50%, thereby decreasing the power to detect a sex-specific effect. Nevertheless, the association is enhanced in males and attenuated in females. Whether or not males in *GPR174/ITM2A* linkage disequilibrium have more severe hyperthyroidism (biochemical and immunological) than those not in linkage disequilibrium requires further investigation. Since genes located on sex chromosomes usually exhibit sex-specific expression under normal conditions (Regitz-Zagrosek & Kararigas, 2017), the transcription level of *ITM2A* in PBMCs needs to be compared after gender stratification.

In summary, our data provide a clear motivation for including the X chromosome in large-scale genetic studies of GD and using the sex-specific GRS for better predictive models. This will become increasingly important as we move further into the era of precision medicine.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHORS' CONTRIBUTIONS

All authors revised the work critically for important intellectual content and gave approval for the final version of the manuscript. HD Song and SX Zhao conceptualized and designed this project. F Sun, RJ Zhang, Y Fang, and QY Zhang took part in the clinical sample collection, DNA extraction, and sample quality control. SX Zhao, CY Yan, YR Ma, and FY Wu analyzed the data. CY Yan, and YR Ma wrote the manuscript and SX Zhao and HD Song critically reviewed the manuscript.

## ETHICS APPROVAL

The employed procedures were reviewed and approved by the local ethics committees of all partner hospitals (Linyi People's Hospital, and the First Hospital Affiliated to Bengbu Medical College), following the principles outlined in the Helsinki Declaration. Informed consent was obtained from all individual participants included in the study.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.